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Size selective predation by *Belostoma oxyurum* (Heteroptera: Belostomatidae) on *Aedes aegypti* (Diptera: Culicidae) larvae

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■ **ABSTRACT.** The aim of this research was to evaluate whether the equations proposed by Holling could predict prey size selection in the *Belostoma oxyurum* (Dufour) / *Aedes aegypti* (L.) system. Prey size choice of *B. oxyurum* on *A. aegypti* as prey was evaluated experimentally. The four larval instars of the prey were exposed (with replacement of as they were consumed) to each of the five nymph instars as well as the adult of the predator for an hour during which attacks were registered. Results were expressed for each predator instar as percentages of attacks for each prey item. All predator instars showed significant differences (Kruskall–Wallis test) in the attack percentages, suggesting a definite preference for some prey sizes. Preferred prey size began with larvae 2 for the 1st instar predator; larvae 3 and 4 for 2nd and 3rd predator instars; and larvae 4 for the 4th, 5th instars and adult predators. A correspondence analysis was performed, which showed a clear association between *A. aegypti* larvae 2 with predator 1st instar; prey larvae 3 with predator instars 2nd to 5th; and prey larvae 4 to predator 5th instar and adult. Preference for greater preys suggested that the prey size range offered was appropriate for the smaller predator instars but too small for the bigger ones. These results were compared with the analytical deduction of the optimal prey diameter for predators with raptorial front leg, proposed by Holling. This deduction overestimated prey diameter (almost 2.8 times larger than observed), probably due to morphological differences in the predator claw; prey morphology; or by the use of artificial prey models *versus* natural preys. The mechanistic approach of the optimal prey size based on morphological measurements does not consider the possibility that the net energy intake is not the highest at the estimated prey size.

KEY WORDS. *Aedes aegypti. Belostoma oxyurum.* Belostomatidae. Culicidae. Predator–prey. Prey choice.

■ **RESUMEN. Predación selectiva por tamaño por *Belostoma oxyurum* (Heteroptera: Belostomatidae) sobre larvas de *Aedes aegypti* (Diptera: Culicidae).** El objetivo de este trabajo fue determinar si las ecuaciones propuestas por Holling podrían predecir la selección de tamaño de presa en el sistema *Belostoma oxyurum* (Dufour) / *Aedes aegypti* (L.). La selección de tamaño de presa por *B. oxyurum* sobre *A. aegypti* como presa fue evaluada experimentalmente. Los cuatro estadios larvales de la presa fueron expuestos a cada uno de los cinco estadios ninfales y al adulto del predador durante una hora; los ataques fueron registrados, y las presas consumidas fueron reemplazadas por frescas. Los resultados fueron expresados como porcentaje de ataques para cada tamaño de presa. Todos los estadios del predador mostraron diferencias significativas (test de Kruskall–Wallis) en los porcentajes de ataque, sugiriendo una definida preferencia por algún tamaño de presa. Los tamaños

de presa preferidos comenzaron con la larva 2 para el primer estadio del predador; larvas 3 y 4 para el segundo y tercer estadio del predador; y larva 4 para el cuarto y quinto estadio y el adulto del predador. Se realizó un análisis de correspondencia, el cual mostró una clara asociación entre la larva 2 de la presa y el primer estadio del predador; la larva 3 de la presa aparece similarmente asociada a los estadios segundo al quinto del predador; y la larva 4 de la presa asociada al quinto estadio y al adulto del predador. El tamaño creciente de presa preferido, llegando a ser la presa de mayor tamaño para los predadores intermedios, sugeriría que el rango de tamaño ofrecido fue adecuado para los estadios más pequeños, pero demasiado pequeño para los más grandes. Estos resultados fueron comparados con la deducción analítica del tamaño óptimo para predadores con primer par de patas raptoras propuesta por Holling. Esta deducción sobreestimó el diámetro de la presa (casi 2,8 veces mayor que lo observado). Las causas posibles son diferencias morfológicas en las patas del predador, la morfología de la presa, y el uso de presas artificiales en vez de presas naturales. La aproximación mecanística del tamaño óptimo de presa basado en medidas morfológicas no considera la posibilidad de que la ganancia neta de energía no sea la máxima al tamaño de presa estimado.

PALABRAS CLAVE. *Aedes aegypti. Belostoma oxyurum.* Belostomatidae. Culicidae. Predador–presa. Selección de presa.

INTRODUCTION

Belostomatids are predacious water bugs distributed almost worldwide, inhabiting various kinds of ponds, usually associated with water plants. *Belostoma oxyurum* (Dufour) is a small bug (overall size 12-14 mm), fairly common in the warmest parts of Argentina, Uruguay and Brazil (Schnack, 1976). These belostomatids are generalised predators, attacking any mobile creature including small invertebrates, fishes and amphibians (Kehr & Schnack, 1991; Cloarec, 1992). Their front legs are raptorial, each formed by the femur and the tibia together with the tarsi and claw, which securely locks the prey and allows the bug to insert its beak to inject histolytic saliva and suck out the digested tissues through the cibarial channel.

Aedes aegypti (L.) is a mosquito which larvae develop in small water containers and ponds. This mosquito is considered one of the most dangerous species, being vector of the yellow fever, dengue and some other diseases. The predatory nature of belostomatids makes them suitable for the biological control of water organisms (Roy & Raut, 1994; Lopez *et al.*, 1997). Previous studies on belostomatids found that these bugs were effective predators on mosquitoes (Dudgeon, 1990; Venkatesan & D'Sylva, 1990; Consoli *et al.*, 1989; Prabakaran, 1992). Literature concerning aquatic predator-prey models is abundant for fishes (Juanes & Domenici, 1994), but scarce for insects and other invertebrates (Chesson, 1989; Kerans *et al.*, 1995; Krishnaraj & Pritchard, 1995; Streams, 1994). Most of the researches about prey selection were descriptive results (Conde-Porcuna & Sarma, 1995; Hunter & Maier, 1994), but some had predicting power (Branstrator, 1998; Greene, 1988; Helgen, 1989).

The optimal foraging theory predicts a maximum energy intake with the minimum cost (handling time) in predator prey systems; thus the predator would select the prey with the highest net energy intake. Holling began in the 60's a series of researches concerning the predator-prey models (Holling, 1965; Holling, 1966). One of the several problems discussed by this author was the prey size and its selection by the predator (Holling, 1964). In this paper Holling offered a mechanistic approach in order to predict the optimum prey size. Mantids *Hierodula crassa* Giglio-Tos, were used as models for the formulation and solution of the problem. Considering the front claw of this insect through simple geometric deductions, he found the optimal diameter of the prey in order to allow its claw to work as a perfect locking mechanism. Holling then carried out a simple experiment to prove his deductions.

Since the morphology of the front claw of the mantids and belostomatids is analogous, the aim of this research was to evaluate whether the equations proposed by Holling could predict prey size selection in the *B. oxyurum / A. aegypti* system as well. The morphology of mosquito larvae fits the assumptions of the equations as their body is approximately cylindrical, and it is an accessible and convenient prey model.

MATERIAL AND METHODS

Belostomatids specimens were collected in Los Talas Pond (Berisso, Buenos Aires Province, and Punta Lara (Ensenada, Buenos Aires Province, 34° 47.05' S; 58° 00.49' W), and were carried to the laboratory in small containers with moist leaves to avoid cannibalism. Adult and different stage nymphs were recognised under a stereoscopic microscope. Bugs were kept in commercial glass flasks (135 cm^3) and fed twice per week. Those bugs used in the experiments were starved before, for approximately 72 hours.

Mosquito eggs laid on filter paper were supplied by the CEPAVE (Centro de Estudios Parasitológicos y de Vectores, CONICET-UNLP). They were submerged in dechlorinated tap water to allow hatching. Four larval instars were recognised and handled with a "Pasteur" pipette. They were fed with Tetra, commercial fish food *ad libitum*. Pupae were excluded from the study because of their different morphology and incompatibility with the assumptions of the equations, as well as their different behaviour (Chockalingam & Somasundaram, 1987; Shankar-Kumar, 1992).

In order to evaluate prey selection, one mosquito larva of each of the four larval instars was placed with a bug in a 135 cm^3 water glass container. A straw was supplied as substratum for the bug. The predator was allowed to feed for one hour, and each attacked larva was immediately replaced by one of the same instar, thus keeping the prey size ratio constant. This arbitrary time lapse, was chosen because in previous tests, this predator was able to attack up to 14 larvae in one hour, and just one or two larvae per day was enough to keep the predator alive. Each one of the belostomatid five instars and adults were tested (1st instar N=9, 2nd instar N=11, 3rd instar N=13, 4th instar N=10, 5th instar N=8, Adult N=8).

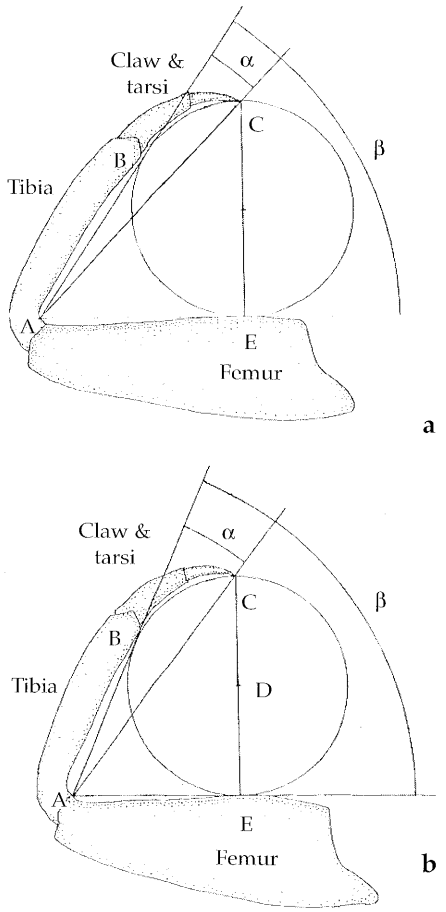

Fig. 1: a, Diagramatic *Belostoma oxyurum* fore leg with α angle = 9°; b, idem with α angle = 14°

Prey selection data per bug was treated as percentage of each instar attacked over the total larvae attacked during the experiment. A non-parametric ANOVA (Kruskal-Wallis and *a posteriori* Dunn's posttest, GraphPad Prism, v.3) was performed for the comparison between the *B. oxyurum* predation rate upon each *A. aegypti* instar. This was performed for each of the five *B. oxyurum* nymph instars as well as for the adult.

One scanning electronic photograph of the front claw of all *B. oxyurum* instars were taken in the MLP scanning electronic microscope service, in order to take the corresponding measurements. Larval mosquitoes of all instars(N=15 for each instar) were fixed with ethanol 70° and their diameter (third body segment) measured with microscope.

The algebraic deduction of the optimal radius of prey was taken from Holling (1964), since *B. oxyurum* has the same attributes as *Hierodula crassa* (Fig. 1).

In order to let the locking mechanism (formed by femur and tibia plus tarsi and apical claw) work correctly, the prey must touch the tip of the apical claw, tibia and femur; and a perpendicular line from the tip of the claw to femur should run through the centre of the prey (D) (see Fig. 1). So the optimal size of the prey can be calculated knowing the length of the tibia and the angle formed between the tibia and the tip of the apical claw, α (complete deduction by Holling, 1964):

$$\tan(\beta-\alpha) = 2\tan\frac{\beta}{2}$$

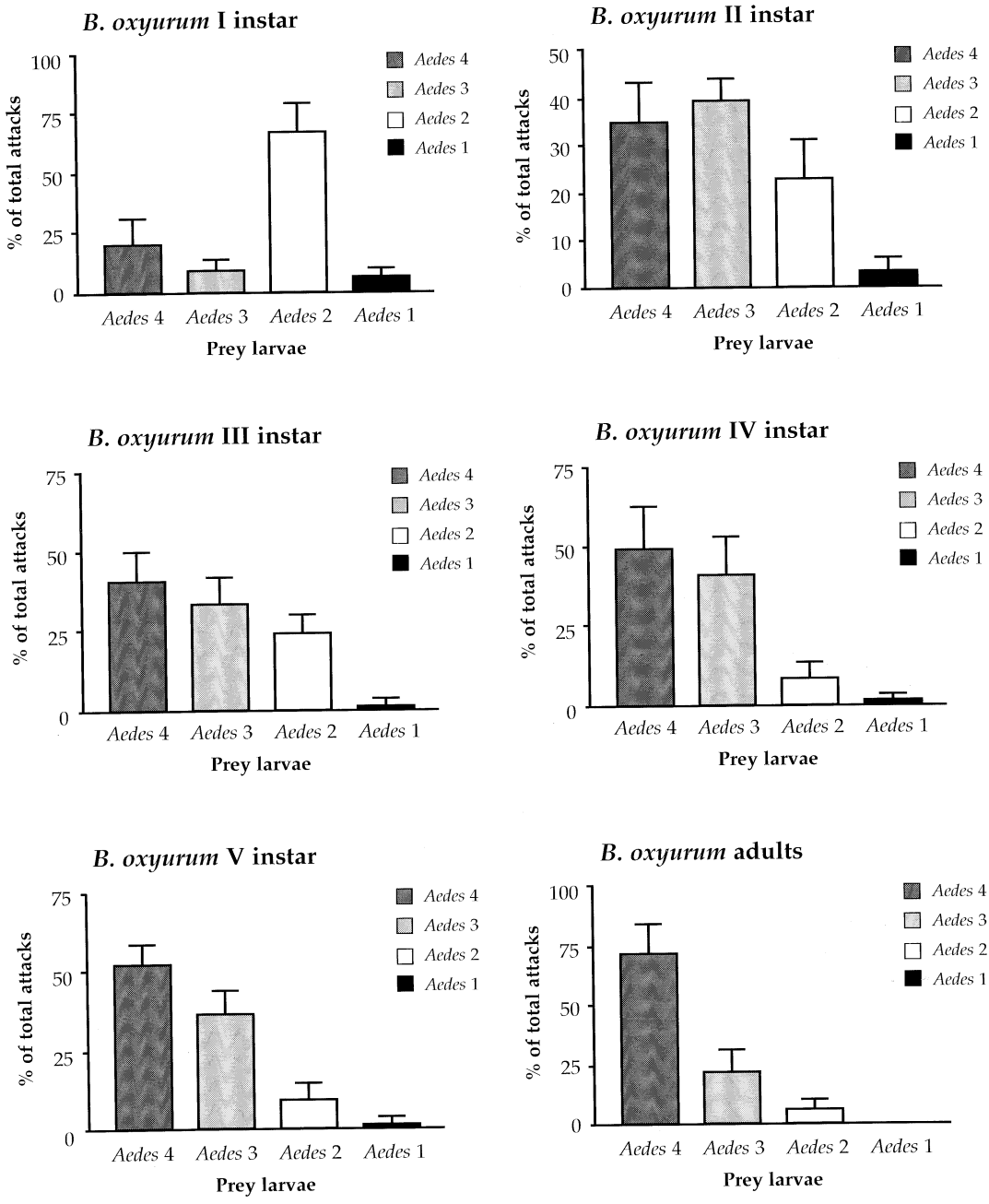

Fig. 2: Mean and SEM of the predation rate of each *B. oxyurum* instars and adult vs. all *A. aegypti* instar larvae.

Then, the optimal angle between the tibia and femur (β), can be deduced from this equation by iterating, just knowing α. An optimisation of this equation from Holling, is here proposed in order to iterate in just one step:

$$\beta = \alpha + \arctan\left(2 \tan \frac{\beta}{2}\right)$$

Once β is calculated, knowing the length of the tibia, the optimal radius of the prey can be found.

$$r = \frac{AC \text{ sen } (\beta - \alpha)}{2}$$

Correspondence analysis between average predation rate of each *B. oxyurum* instar with all the prey larvae was performed (Statistica v. 2000); standarization of coordinates was through rows and columns profiles.

RESULTS

Figure 2 shows the mean and SEM (standard error of the mean) of the predation rate of each predator instar vs. all available prey instars (mean and SEM used just for better visualization of the results).

Predation rates of all *Belostoma oxyurum* instars for each *Aedes aegypti* larvae showed significant differences at least in one pair of comparisons (all six comparisons were significant at an $\alpha = 0.005$). Each *Belostoma oxyurum* instar showed stronger preference for some prey instars over others. Dunn's post test for the choice of predator instar I for each prey larvae: larvae 2 vs. 1, and larvae 3 vs 2 were significantly different; predator II and III: larvae 3 vs 1 and 4 vs 1; predator IV: larvae 4 vs 1; predator V and adult: larvae 4 vs 1 and 4 vs 2, all significant at $\alpha = 0.05$. Those comparisons not stated were not significant. In some *B. oxyurum* instars preference was evident for one prey instar over the rest. This is the case of B. oxyurum 1st instar, which preferred *Aedes aegypti* larvae 2 instar as evidenced in Fig. 2, even though only the choice for larvae 1 and 3 were statistically different. *B. oxyurum* 2nd and 3rd instars, would prefer *A. aegypti* larvae 4 and 3. For *B. oxyurum* 4th and 5th instars and adult, the choice for larvae 4 was significantly different from that of *A. aegypti* larvae 1 (for *B. oxyurum* 4th instar) and from larvae 1 and 2 (adult and 5th instar, Fig. 2).

Measurements taken on *Belostoma oxyurum* front legs, toghether with deductions of optimal prey diameter are shown in Table 1. Mean and SD of prey diameter were: larva 4= 0,8 ± 0.07; larva 3= 0.5 ± 0.03; larva 2= 0.28 ± 0.03; larva 1= 0.17 ± 0.02.

Correspondence analysis between average predation rate of each *B. oxyurum* instar with all the prey larvae showed that total inertia was 0.3 (Chi square value = 183.4, 15 degrees of freedom, $P<0.00001$) The first canonical dimension accounted for 87.8% (Eigenvalue = 0.26) of inertia, and the second one for 11.8% (Eigenvalue = 0.03). There was a clear association between *A. aegypti* larvae 2 with predator 1st instar, being these far away from the rest. Predator instars 2nd to 5th were similarly associated to prey larvae 3, even though *B. oxyurum* 2nd and 4th were statistically nearer than the others. Predator 5th instar was about the same distance from either *A. aegypti* larvae 3 and 4. *Belostoma oxyurum* adult was associated to *A. aegypti* larvae 4 being far away from any other point.

Table 1: Optimal diameters found through Holling's equation.

Instar	Measured α angle	Length of tibia	calculated β angle	Calculated r optimal prey radius
I	16°	1.043 mm	70.94°	0.427 mm
II	14°	1.391 mm	66.84°	0.551 mm
III	14°	1.619 mm	66.84°	0.645 mm
IV	14°	2.136 mm	66.84°	0.853 mm
V	14.5°	2.272 mm	67.88°	0.911 mm
Adult	15.5°	2.712 mm	69.94°	1.022 mm

DISCUSSION

Experiments suggest a growing prey size preference that would begin with larvae 2 for *B. oxyurum* 1st instar; 2nd and 3rd instars would prefer a bigger prey size (larvae 3 and 4) and from the 4th instar to the adult would prefer mainly the biggest prey size over the smallest ones. Due to the facts that the biggest prey available was the larvae 4, and that from the 4th predator instar to the adult the biggest prey was prefered, it is possible that the biggest predators would choose the biggest available prey size "by default", but if they were available, the predator would choose even bigger preys, following the tendency observed in the smaller predators. This assumption lead us to think that the prey size range offered was appropriate for the smallest predator instars, but was too small for the biggest ones.

The correspondence analysis support the preference of the 1st instar predator for the prey larvae 2, and of the adult for the larvae 4, as specific associations different from all others. The predator's instars 2nd to 5th were in an intermediate point between prey larvae 3 and 4.

Holling equation overestimated the optimal prey size for *B. oxyurum*, *i.e.* the first instar should have had higher preference for *A. aegypti* larvae 4, but instead it chose intermediate sized preys. The experimentally preferred prey was almost 2.8 times smaller than the estimated optimal prey diameter for *B. oxyurum* first instar, and the following predator instars should have had higher preference for the biggest prey size, but this was not the case.

The overestimation of Holling equations for *B. oxyurum* could have had several causes. One of them could be prey morphology, which was vermiform, but maybe too long and therefore too big to handle and eat. For example *Belostoma oxyurum* 1st instar which attacked a *A. aegypti* larvae 4, were dragged away for a few minutes until the prey was subdued, and one of them spent almost an hour eating that single prey. Biggest predator instars attacked the biggest available prey, and possibly they would have preferred much bigger preys, if they had been available.

Another reason could have been a morphological difference in the predator here studied and the one chosen by Holling. The tibial claw tip of mantids is fixed, being an overdeveloped spine,

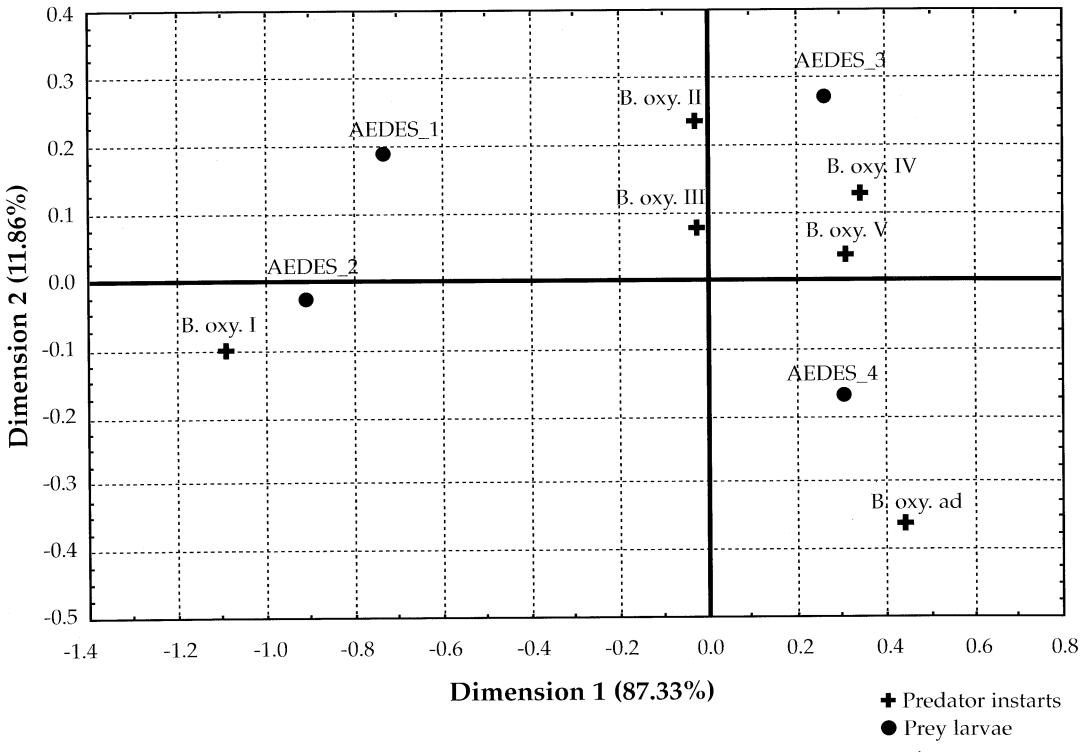

Fig. 3: Correspondence analysis performed of predation rates of each *B. oxyurum* instars and adult (*B.oxy.*I to *B.oxy.*Ad.) vs. all *A. aegypti* larvae (*Aedes* 1 to *Aedes* 4).

but the tip of the claw mechanism of belostomatids is very different. In this family, the tibia has no disproportionate spines and its whole inner surface is covered by short and tough setae; the tarsi follow the line of the tibia and carry the claw. The tarsi are articulated with the tibia and therefore they can change the alfa angle in the leg, as well as the measured tibial length. This feature would allow this predator to display a range rather than a single alfa angle, thus enhancing its versatility. The measured range in the 3th instar *B. oxyurum* for the a angle was from 9° to 14°, and the tibial length ranged from 1.74 mm (for α 9°) to 1.62 mm (for α 14°) (Fig. 1). But the optimal radius ranges only a few decimals (from 0.644 to 0.645 mm). This negligible difference is possibly due only to differences in the decimals truncating. Even though the a angle varied more than 50%, the result remained the same. The stability in the results was due to the equation form itself, because if it is assumed that the prey must touch at least three points, namely the femur, the tibia and the tip of the claw, a bigger alfa should force beta to increase too and displace the contact place in both tibia (B) and femur (E) closer to the vertex of the angle. In this case AE and AB ranged from 1.14 mm to 0.98 mm for $\alpha = 9°$ and $\alpha = 14°$ respectively. The inverse should happen if a decreased. Thus the sine of difference between the angle β and α should remain almost constant.

Considering the results of Holling equation exclusively, the function of this articulation would not be the change in the optimal prey size. The function remains speculative, and could just have some importance in the moment of the strike of the claw against the prey, allowing the predator more chance of locking the prey securely and faster, as it is shown by the various observations of the power and versatility of these predators (Cloarec, 1989; Cloarec, 1991b). Also could it have some importance in multiple prey item handling (Cloarec, 1991a).

The question is whether Holling equation should predict the optimum prey size accurately. In this mechanistic deduction, only few morphological traits are considered, and the outcome of the deduction is just a measure. So, the optimum prey size predicted could probably not harvest the highest net energy intake. Other traits should be considered in order to find out the optimum prey size, such as dry weight and energy of the prey, and costs to the predator.

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